

# Effects of Levamisole Treatment in Cancer Patients

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**Abstract.** Twenty-six controlled prognostic evaluations of the adjuvant use of levamisole in cancer are reviewed. The results favor intermittent administration of levamisole in a dosage that is adapted to the patient's weight or body surface. Early treatment is indicated, but synchronous treatment with cytotoxic therapies is to be avoided. The best results have been achieved in advanced but still potentially curable patients. Major toxicity occurs very seldom and measures are suggested to further characterize the few patients who are at risk of developing allergic agranulocytosis, a potentially life-threatening side effect. (*J Rheumatol* 5 (Suppl 4): 123-134, 1978)

**Key Indexing Terms:**

CANCER

IMMUNOTHERAPY

LEVAMISOLE

The scope of the present overview will be restricted to the clinical effects of levamisole treatment in cancer patients. Moreover, as the experience in animal cancer models has indicated that levamisole is to be used as an adjunct to classical anti-cancer treatment<sup>1</sup>, only controlled clinical trials of the adjuvant use of levamisole in cancer patients will be discussed. Pilot clinical studies, using levamisole as a monotherapy, tend to confirm the animal data in that such therapy has produced no or only marginal benefit.

## THE AVAILABLE EXPERIENCE

This overview is concerned with results received up to May 1978 from 26 controlled

clinical studies evaluating the use of levamisole as an adjuvant treatment. Some of these data are still very preliminary. In all, 1474 levamisole-treated patients are included in these trials as well as about 1600 controls. Ten of these studies were conducted in Europe, 10 in North America, three in South America, one in Japan, one in New Zealand, and the remaining study is a multicentre pilot study initiated in Europe and South America.

Two studies are concerned with various types of cancers (305 patients treated with levamisole), but the other 24 studies are focused on a more homogeneous group of levamisole-treated patients, i.e. breast cancer in seven trials (382 patients on levamisole), lung cancer in four studies (199 cases), colorectal and/or other digestive cancers in three (192 patients), leukemias or lymphomas in five (262 cases), malignant melanoma in two (163 patients), head and neck carcinomas in two (38 patients), and bladder cancer in

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one (29 cases). Levamisole has been given as an adjunct to surgery in six studies (330 patients), to irradiation therapy in four studies (276 patients), and to cytostatic chemotherapy in 13 studies (594 patients); in one study (with 12 patients on levamisole) the primary treatment was surgery and/or radiotherapy and in the remaining two trials (262 levamisole patients) different types of primary treatment were used.

Levamisole effects are not directly evaluable in two studies<sup>2,3</sup> as the drug was combined with BCG in both cases and also with sodium warfarin in one of them<sup>2</sup>, whereas no control group was available that had been treated with these associated agents alone. In another study<sup>4</sup>, levamisole is combined with (intrapleural) BCG as well, but in this case a control group, treated with BCG only, is being studied.

## THE RESULTS

As mentioned, nine reports are of an early evaluation of a study. Therefore, the present section will first focus on the 17 reports on more advanced trials and the preliminary reports will briefly be dealt with in a second part.

### A. THE MORE ADVANCED STUDIES

Fifteen studies deal with one type of cancer; one deals with different cancers of the digestive tract where various types of primary treatment have been used<sup>5</sup>; and one with a wider spectrum of malignancies but all treated with the same primary therapy, *i.e.* irradiation<sup>6</sup>.

All studies have produced a trend to the advantage of levamisole, at least in a subgroup of patients (see further), that reached the level of statistical significance in 13 of the 17 studies. In one more study<sup>7</sup> the data are of borderline statistical significance and in the other three studies, all of them using cytostatic chemotherapy as a primary treatment modality, the subgroup of respond-

ing patients was not evaluated separately (two studies in reference 8) or the subgroup was so small (only nine patients in the control group) that a lengthening of the median survival time from 19 to 35 weeks fell short of reaching statistical significance<sup>8</sup>.

The results, provided by these 17 studies clearly suggest several aspects which have to be taken into account when levamisole is used in clinical cancer. These aspects are discussed below.

### 1. Dosage Regimen

In all but one study<sup>10</sup> levamisole treatment was given intermittently. There is no indication that continuous treatment would be superior to intermittent therapy with levamisole. Intermittent treatment has been given for two to three consecutive days every week or every fortnight, though in some cases a few treatment courses were omitted to avoid a synchronous administration with cytostatic agents given in cycles<sup>8,11</sup> or with BCG<sup>3</sup>. It seems fair to conclude that there is no difference in efficacy between the weekly and the fortnightly intermittent schedule. Therefore, the former treatment scheme may be favored as more practicable.

The dose used was either fixed at 150 mg per day<sup>2,5,6,7,9,11-15</sup>, usually given as 50 mg *t.i.d.*, or tailored to the weight of the patient, in which case 2.5 mg/kg was given<sup>3,8,16</sup> or to his body surface area, *i.e.* 100 to 120 mg/m<sup>2</sup><sup>10,17</sup> which is slightly in excess of 2.5 mg/kg for most patients. Since the animal data do suggest that a certain dose level is to be met in order to obtain an optimal efficacy with levamisole in cancer<sup>1</sup> and since a double-blind placebo-controlled study of levamisole in children with recurrent infections has produced evidence that the therapeutic results may be critically dependent upon the dosage of levamisole<sup>18</sup>, it is important to know whether the individually adapted dose is superior to the fixed one. Clearly, this question is only relevant

for patients weighing more than 70 kg as in the other patients the fixed dose is almost identical to the 2.5 mg/kg dose. A direct comparison of the two dose levels in patients weighing more than 70 kg is not available, so we have to infer from the results obtained in this subgroup of patients with the fixed 150 mg dose. Most studies however have been performed on too small a scale (i.e. less than 50 patients in the levamisole group) to allow a meaningful analysis of this aspect<sup>2,11,13-15</sup>. In one more study, involving chemotherapy as a primary treatment, the number of patients responding to the latter treatment is less than 50 as well, again precluding a reliable evaluation of this factor<sup>9</sup>. Two more studies appear less suited for such purposes as they deal with a heterogeneous patient group<sup>5,6</sup>. Moreover, in one of these studies the response to the primary treatment (which was irradiation) is not given either<sup>6</sup>. Therefore, only two studies, one in malignant melanoma<sup>7</sup> and the other in bronchogenic carcinoma<sup>12</sup>, seem to be suitable for a separate analysis of the effects in patients weighing more than 70 kg. Unfortunately, no data are available on the weight of the patients in the former study<sup>7</sup> and such a separate analysis has not been made either. On the other hand, this aspect has been very carefully evaluated in the bronchial cancer trial<sup>12</sup>; this study is a three-centre evaluation of levamisole used as an adjunct to surgery. A significant superiority to placebo was found with levamisole in patients weighing  $\leq 70$  kg whereas there was no benefit from levamisole in patients weighing more. In order to rule out chance as the factor producing this difference, the data were analyzed separately for each centre and also for the first half of patients entering the study and those selected in the second part of the trial. Each of these analyses produced a similar trend in that levamisole was only clearly superior to the placebo in those patients whose weight was

70 kg or less. This analysis leads to the conclusion that a bodyweight- or body surface-adjusted dosing is to be preferred over the fixed dosage regimen.

When is adjuvant treatment with levamisole to be started? From the theoretical point of view, the answer is as early as possible. Immunotherapy is primarily designed to eradicate small numbers of malignant cells as the host defense mechanisms are known to be able to keep in check only a limited tumor load. It follows that, the larger the tumor residue after primary therapy and the faster the growth rate of the cancer, the more critical an early initiation of treatment will be. The available clinical data offer only a few facts to confirm or to reject this thesis. The main reason for this is that levamisole has been started at a fixed time as compared with the time of administration of the primary treatment in several studies, and in the other studies this aspect has usually been overlooked in analyzing the data. Nevertheless, some data do point to the importance of timing in the initiation of levamisole treatment. In a laryngeal carcinoma study<sup>16</sup>, it was found that none of the levamisole-treated patients relapsed within the first year after primary treatment except one single patient who had a relapse after approximately seven months: the start of levamisole treatment had been delayed till 49 days after this initial therapy, in contrast to the other levamisole patients in whom the treatment was started within the first 19 days (Mussche: *personal communication*). In another study, involving patients with resectable colorectal cancer<sup>14</sup>, treatment with levamisole was started when the patients left the surgical department. The median delay from surgery to initiation of immunotherapy was 17 days; in those patients in whom this therapy was started within the first 17 days, cancer mortality was clearly reduced one and a half year after the resection, whereas no beneficial

# SURVIVAL 18 MONTHS AFTER RESECTION

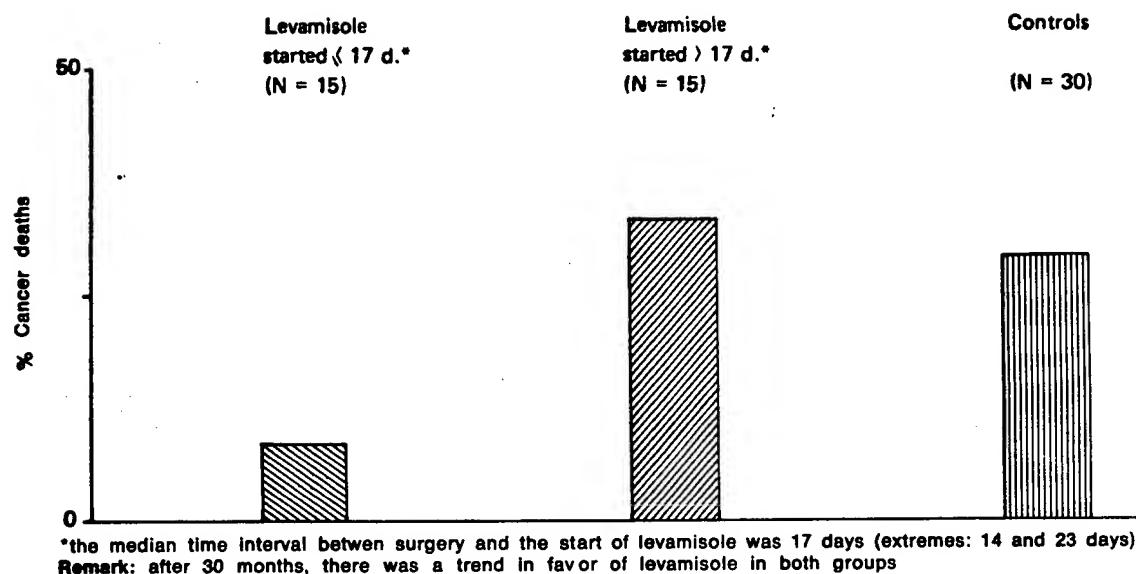


Fig. 1. The importance of an early start with levamisole after surgery for colorectal cancer.

trend was found in those in whom the start was delayed till more than 17 days after the operation (Figure 1). Nevertheless, the survival rate in the latter group one year later, *i.e.* after two and a half years, was also ameliorated by the immunotherapy. These findings are consistent with the idea that an early start of levamisole treatment is particularly critical for those patients who will relapse early and, therefore, probably those patients who have a relatively large, though clinically undetectable, tumor residue after the operation and/or those who have a fast-growing type of cancer. In order to avoid this problem, it seems wise to start levamisole treatment shortly before surgery. There is no indication, from the few studies where this has been done, that there is risk involved, regarding pre- or post-surgical complications. Regarding other treatment modalities, however, there is actually experimental evidence<sup>19,20</sup> that the concomitant administration of levamisole and cytotoxic treatment

may be deleterious. Therefore, it seems desirable to start levamisole treatment after the completion of irradiation treatment — this has been the case in most studies of that type — or to have the administrations of levamisole interspersed between the courses of chemotherapy, if given intermittently, or to delay its start till the termination of aggressive induction chemotherapy: these schedules have been used in most chemo-immunotherapy studies.

## 2. Patient Eligibility

Our classical methods of classifying malignant diseases are largely based upon the organ from which the tumor originates and upon the histological appearance of that tumor. So far, these criteria have not proved to be useful in making a distinction between those cancers that are susceptible to levamisole therapy and those that are not, as judged from the results found in such a wide range of malignancies and from the observation

that the effects obtained in bronchogenic carcinoma could not be related to the histological type of the cancers studied<sup>12</sup>. This should not be surprising as the reaction of the host to his cancer is not reflected by these classification methods and since the histological classification largely ignores the membrane characteristics whereas the membrane is the part of the cancer cell that is recognized by the host defense mechanisms. Nevertheless, it seems probable that some cancer-bearing patients will benefit more than others from adjuvant treatment with levamisole. The remainder of this section will deal with the findings that may be helpful in defining such subcategories of patients.

It has been noted in five studies<sup>5,7,12,14</sup> so far, that the difference (always to the advantage of levamisole treatment) between the levamisole-treated patients and the control group was more pronounced in those patients who had more advanced tumors before initiation of the primary therapy. This would indicate that the latter type of patient is the more suitable for levamisole treatment, provided that the cancer still proves susceptible to classical anti-cancer therapy. If true, this could find its explanation in the fact that more advanced tumors are associated with a higher probability of immune deficiency, and that levamisole, as an anti-anergic agent, restores the latter type of deficiency. However, an alternative explanation should be borne in mind, i.e. that patients with an early cancer (usually Stage I) often have a very good prognosis; especially during the first few years following adequate primary treatment, even without immunotherapy. Therefore, it will take a few more years to discover whether levamisole is completely or almost completely ineffective in Stage I patients or whether the present trend is merely due to an insufficiently long follow-up of such patients.

The immune status of the host may be

expected to be the most reliable factor predicting levamisole effectiveness. However, the problem here is to establish appropriate criteria and there is still a long way to go in that regard. Nevertheless, a simple measure like the absolute lymphocyte count before treatment was found in two independent studies<sup>14</sup> (and Mussche: *personal communication*) to be a helpful factor in characterizing a subpopulation with a high risk of recurrence if left without immunotherapy, but with an improved chance of survival if treated with levamisole. Such observations strengthen the hope that relatively simple immunological measures can be found which will be helpful in further defining the appropriate group of patients for therapy.

### 3. What results may be hoped for if levamisole is given to cancer patients?

Although studies using levamisole as a monotherapy are beyond the scope of the present overview, it seems fair to state here that the results of such trials have been disappointing. The view is widely accepted that, in order to obtain effects from immunotherapy, one should reduce the tumor load first. This rule appears to hold true since the general feeling is that levamisole treatment should aim at consolidating anti-cancer effects obtained by other therapies. If there is nothing to consolidate, i.e. when the classical anti-cancer treatment has been ineffective, there is no evidence that levamisole will be helpful to the patient. It is of interest in this regard that chemo-immunotherapy studies in animals, using five different types of cytostatic drug and three types of immunotherapy (one of these being levamisole), have revealed that the prolongation of remission with immunotherapy was related to the effectiveness (as measured by the nadir of the tumor size) of the chemotherapy<sup>21</sup>. Such data provide a pharmacological basis for the clinical experience using chemo-immunotherapy that has shown that immunotherapy

(usually BCG in these cases) is capable of prolonging the duration of remission as well as the survival of those patients who respond to the chemotherapy<sup>22</sup>. Returning to levamisole, three groups of investigators have separately evaluated the effects in the patients who responded to the cytostatic chemotherapy<sup>9,11,17</sup>; in each case it was found that a beneficial overall trend regarding survival was due to the effect in the group of the responders whilst there was no benefit from levamisole in those patients whose tumor kept growing in spite of the chemotherapy. It seems therefore mandatory to make a separate analysis of the results obtained in the responders when chemo-immunotherapy trials are evaluated; the lack of such a differential analysis might explain why no significant prolongation of survival was found in spite of an apparent trend in a couple of such studies<sup>8</sup>. The same may hold true for the adjuvant use of levamisole in irradiated patients described in a study<sup>6</sup> involving different cancer types submitted to irradiation therapy. The best results in this study were obtained with those malignancies that are known to be usually quite susceptible to radiotherapy, *i.e.* those of the breast, the head and neck area, and the cervix, whereas the results obtained in lung cancer, probably the most radioresistant cancer in that study, were that more than three quarters of the patients died within the first year in both the levamisole and the control group. Unfortunately, no data on the result of the radiotherapy are available from that study so that this important aspect cannot be evaluated.

The results obtained with levamisole in experimental cancers<sup>1</sup> suggest that this drug may be more effective in preventing dissemination than in controlling the growth of the primary tumor. In most clinical studies, such a preferential inhibition of metastasis has not been looked at separately. Nevertheless, in the two investigations where

this aspect has been studied, the findings are in keeping with such a preferential effect<sup>12,13</sup>.

Finally, the addition of levamisole to cyclic chemotherapy has resulted in some instances in an increased remission rate<sup>8,11,23</sup> although such an increase could not be found in other studies<sup>9,17</sup>. Perhaps the remission rate can be increased by adding levamisole to certain treatment schemes with cyclic cytostatic chemotherapy. Clearly, however, much more experience of this sort is needed to evaluate which factors may play a role, although it seems likely that the immune status of the patient, the type of cytostatic agent, the treatment scheme, and the degree of marrow toxicity may all be influential in this context.

Are there any other effects which one can expect from levamisole treatment? As reported by Lods and co-workers<sup>24,25</sup>, levamisole treatment may enhance bone marrow reconstitution in patients treated with cytostatic chemotherapy and, in line with these findings, the increased response rate observed in one chemo-immunotherapy study could be partly or entirely explained by the fact that the levamisole-treated patients had received higher amounts of chemotherapy and had better tolerated this treatment<sup>11</sup>. It is also conceivable, that levamisole treatment may decrease the incidence of serious infections in immunologically deficient cancer patients. So far, however, such an effect of levamisole has not been the subject of a carefully designed study.

*In summary*, where levamisole is used, treatment should be intermittent, adapted to the patient's weight or to his body surface area, and started as early as possible, though a synchronous treatment with cytotoxic treatment modalities is to be avoided. Advanced though still potentially curable patients seem to profit most from levamisole treatment as do patients with a deficient immunity, but

there is a problem in establishing useful criteria to measure this deficiency. In appropriately selected patients, levamisole appears to consolidate the effects obtained by other anti-cancer therapies, it may preferentially inhibit metastasis and, in some treatment schemes, may increase the remission rate induced by chemotherapy.

#### B. THE EARLY-STAGE REPORTS

These studies are in too early a stage to allow any conclusions to be drawn, either because the absolute number of patients or the number of patients responding to the primary treatment is limited, i.e. fewer than 25 patients treated with levamisole or responding to the initial treatment<sup>26-28</sup>, or because duration of follow-up is too short<sup>4,29-32</sup> or because the follow-up data are not yet available<sup>23</sup>. Nevertheless, it may be noted that none of these studies has so far produced a trend to the disadvantage of levamisole, whereas several of them have shown results which may be hopeful. If one deliberately takes an optimistic standpoint, one may point to a higher response rate in a subgroup of the patients in one study<sup>23</sup> or, to a slight tendency to have later recurrences or deaths with levamisole after primary treatment<sup>26,27,29,30-32</sup>, thus leaving only two studies<sup>4,28</sup> with almost no trend at the present time.

Further patient accrual and prolonged follow-up may yet show an advantage of levamisole in the majority of these trials. One can but hope that the investigators in these studies will take into account the trends that came out of the more advanced clinical evaluations, as summarized at the end of the previous section, when they decide to analyze the data. In this context, the experience with the malignant melanoma study in San Francisco<sup>7</sup> has been very illustrative and instructive. When that study was analyzed in an earlier stage, no trend could be found

although the patient accrual had already reached 132 by then<sup>33</sup>. More than one year later, when another interim analysis was performed<sup>7</sup>, a slight trend was found favoring levamisole treatment. Moreover, when the analysis was stratified according to the stage, no benefit from levamisole could be evidenced in Stage 1 where the prognosis was still very good in either study group, whereas levamisole appeared to be superior to the placebo in Stage 2 patients (75% survival time between 9 and 10 months in the placebo group and 18 to 19 months in the levamisole group). Certainly, it would be of interest to see a further analysis of these data according to the weight of the patients (a fixed 150 mg dose has been used), along the immune status at the start and possibly other factors.

#### II. Acceptability

Levamisole is intended to be used as an adjuvant therapy and not as a remission-inducing agent. This difference seems to be very relevant if one considers the acceptability of such a treatment, as the clinician will more readily accept toxicity if an agent is concerned which may drastically reduce the tumor bulk than for a therapy that is merely to be used as an adjunct. The acceptability of levamisole, therefore, ought to be discussed while keeping in mind the most actively evaluated modalities of adjuvant therapy, i.e. other types of immunotherapy and cytostatic chemotherapy. A direct comparison with these other modalities is not available, but the side effects of the latter are pretty well known and this makes a direct comparative evaluation of the adverse effects less mandatory.

In general, levamisole has been well tolerated as only very few patients have stopped their treatment because of side effects. Several authors<sup>13,15,17,28</sup> state that levamisole toxicity was negligible in their study. Moreover, placebo-controlled double-

blind studies have shown that side effects are regularly seen in this type of patient with a placebo<sup>12,29,32</sup> which underscores the importance of including a placebo in such trials. As in non-cancer studies, the only major side effect was a reversible agranulocytosis, which was reported in six patients belonging to the controlled studies reviewed here. Five of these patients participated in a Finnish study of advanced breast cancer<sup>8</sup>, the other patient belonged to a bladder cancer trial<sup>32</sup>. As judged from this experience, the incidence in cancer patients of levamisole-induced agranulocytosis would be about 0.4% (six out of 1474 patients). Levamisole was discontinued in one other patient, belonging to another study in advanced breast cancer<sup>2</sup>, because of leucopenia, but this condition should not be considered equivalent to or even prodromal to agranulocytosis<sup>34</sup>. Apart from this, a higher incidence of agranulocytosis in women as observed in this overview is in agreement with the higher incidence in females reported in general treated with levamisole<sup>34</sup>.

#### DISCUSSION

The available controlled experience with the use of levamisole as an adjunct in the treatment of cancer, as reviewed here, is suggestive of a true therapeutic effect. Also, it may be expected that several of the trials, which only have produced very preliminary data so far, will eventually show a superiority of levamisole to no treatment. From data already available, it may be tentatively suggested that the adjuvant use of levamisole is especially effective in those patients who are known to be at risk of developing recurrent disease after they have undergone clinically effective anti-cancer treatment. In particular, one would think here of patients who have been treated for the first time because of lung cancer, mammary carcinoma, colorectal cancer, or leukemia. If such patients receive an adequate treatment with levamisole, as discussed in the text, there

is a good chance that the effect obtained by the primary treatment will be maintained for a significantly longer time than if they receive no further treatment. Also, this beneficial effect would not be expected to carry with it too high a level of toxicity.

Regarding toxicity, agranulocytosis in the patients discussed here has been reversible. As far as can be judged from this and other material<sup>34</sup>, levamisole agranulocytosis in cancer patients occurs more readily in female patients and in subjects who carry the HLA-B27 antigen. One of the patients in the Finnish breast cancer study<sup>8</sup> also had rheumatoid arthritis (RA), another factor known to predispose to levamisole agranulocytosis. Still, one may hope that other predisposing factors will be found and they should certainly be looked for. In this connection, attention should be paid to:

1. *Geographic aspects.* Five of the six cases, discussed in this paper, came from Finland. This recalls the fact that agranulocytosis with clozapine, a neuroleptic drug, was found to be 10 times more frequent in Finland than in other countries<sup>35,36</sup>. No explanation is available for the latter discrepancy. This Finnish experience with breast cancer, on the other hand, is somewhat counterbalanced by the absence of agranulocytosis amongst 31 acute leukemia patients treated with levamisole in the same country<sup>15</sup>.
2. *Abnormalities of the granulocyte membrane.* RA is a well known predisposing factor to agranulocytosis with levamisole, but the majority of patients suffering from this disease regularly take anti-phlogistic agents, which, apart from causing agranulocytosis themselves in some patients, are known to interfere with leukocyte chemotaxis and/or phagocytosis, two functions in which the membrane of the cell is involved. Other or similar (structural?) changes of the leuko-



cyte membrane may occur in cancer as well<sup>37,39</sup> and there is some indication that such abnormalities are more frequently found in patients with metastases<sup>39</sup>. In this context, it may be mentioned that five of the patients, discussed here, had advanced cancer.

3. *Serum factors.* Such factors could be substances that inhibit leukocyte functions as observed in cancer<sup>38,39</sup> and as discussed above, or, in general, factors that are usually a sign of a disregulated immunity such as the rheumatoid factor — this factor is not only found in RA but may also be encountered in bladder cancer<sup>40</sup> — or cryoglobulins as observed in one non-rheumatological patient who developed agranulocytosis with levamisole<sup>41</sup> and as is present in a substantial proportion of patients suffering from RA<sup>42</sup>. The latter cryoglobulins might also, at least partly, provide an explanation for a possibly higher incidence of levamisole-induced agranulocytosis in a sub-arctic climate as in Finland.

4. *Drugs used concomitantly.* Antiphlogistic drugs have already been referred to above in view of their effects on the leukocyte membrane. Some cytostatic agents are known to deteriorate certain granulocyte functions, such as phagocytosis, as well. One such example is methotrexate<sup>43</sup>. This may be relevant to the observation that three out of five children with acute lymphatic leukemia who were being treated with polychemotherapy — one of the drugs used was methotrexate — and levamisole, developed an agranulocytosis<sup>44</sup>, although maintenance treatment with methotrexate was also given to the 31 levamisole patients of a leukemia study<sup>15</sup> in which no cases of agranulocytosis occurred. On the other hand, four cases suspected of allergic reactions to I.V. methotrexate or a contaminant of it have been reported<sup>45</sup> and two of the

cases were also under treatment with BCG: the presence of degradation products or other contaminants could, therefore, be an explanation why the combination of methotrexate with levamisole causes allergic agranulocytosis in some trials but not in others.

There is a tendency to give levamisole only once a week in an attempt to diminish allergic side effects and to detect agranulocytosis in time. One could consider extending this trend to the cancer field, as well. From the scientific point of view, it would certainly be of interest to find out whether such a dosage regimen would be effective in cancer. However, it took six years of active clinical research to come to the tentative conclusions that we now have regarding the efficacy of levamisole in cancer. In view of the fact that the incidence of agranulocytosis in cancer is undoubtedly low, that this side effect has always been reversible, and that the drug, in general, appears to help patients without causing major problems, it seems reasonable, at the present stage, to give priority to the further evaluation of the efficacy that can be expected from the treatment schedule used so far. This approach, in all probability, will provide the earliest answer to the question of what a cancer patient may or may not expect from treatment with this drug. Obviously, if a certain category of cancer patients turns out to be very much at risk (as defined by one or more factors discussed above), trials evaluating the effects of a one-day-per-week treatment may be initiated in such patients. In the latter case, also, it would be very helpful to know whether the optimal dose of 2.5 mg/kg per day holds true if levamisole is given in one intake per day instead of dividing this dose over three intakes. The answer could be found in the material collected in the San Francisco melanoma study<sup>7</sup> in which levamisole (150 mg per day) was given as a single dose on the treatment days.

Table 1. *Controlled Studies of Levamisole as an Adjunct in Cancer Therapy*

Reference*	Diagnosis	Primary treatment	Types of control	Number of patients levamisole controls	Remarks
12	Bronchogenic carcinoma, resectable	Surgery	Randomized, placebo-treated	96	final report
3	Squamous cell lung cancer, advanced	Irradiation therapy	Randomized	I. 16 II. 17	no pure levamisole arm
13	Breast cancer, Stage III	Irradiation therapy	Parallel group by alternating	24	—
17	Breast cancer, Stage III	Irradiation therapy	Historical	33	—
17	Breast cancer, metastatic	Cyclic FAC chemotherapy	Historical	70	—
11	Breast cancer, metastatic	Cyclic FAC chemotherapy	I. no immunotherapy	127	—
8	Breast cancer, advanced	Cyclic FAC chemotherapy	II. BCG	I. 44 II. 105	—
8	Breast cancer, advanced	Cyclic FAC chemotherapy according to Hanham	Randomized, placebo-treated	31	—
2	Breast cancer, advanced	Cyclic AOS chemotherapy	I. Parallel, non-randomized	25	—
2	Breast cancer, advanced	Chemotherapy (3 drugs) + Prednisolone	II. Historical	I. 19 II. 24	—
14	Colorectal, resectable	Surgery	Randomized, placebo-treated	24	—
15	Digestive cancers, all stages	Different types	Randomized	22	no pure levamisole arm
15	Acute leukemia, 1st complete remission	Maintenance chemotherapy with 6 MP + MTX	Matched parallel group	30	—
10	Acute lymphocytic leukemia in complete remission	Maintenance chemotherapy with 4 agents	Historical	143	—
7	Malignant melanoma, resectable	Surgery	Randomized	31	—
9	Malignant melanoma, disseminated + refractory	Surgery	Randomized	111	—
16 + p.c.	Laryngopharyngeal carcinoma	Actinomycin-D	Randomized, placebo-treated	103	—
6	Various, i.e. breast head and neck cervix lung	Surgery and/or radiotherapy	Sequential	60	—
6	Various, i.e. breast head and neck cervix lung	Irradiation therapy	Randomized, placebo-treated	12	—
28 + p.c.	Bronchogenic carcinoma, advanced	Cyclic MACC chemotherapy	Parallel group by alternating	186 i.e. 58	12 62
4	Lung cancer, resectable	Surgery	Randomized, placebo-treated	24	37 18 24
26	Colorectal, metastatic	Cyclic FU-M-BAF chemotherapy	Randomized:	I. 31	early report
27	Acute myeloid leukemia, 1st complete remission	Cyclic maintenance chemotherapy with 8 different agents	II. C. parvum	I. 28	early report
23 + p.c.	Non-Hodgkin's lymphoma, advanced	Cyclic CHOP-Bleo chemotherapy	Randomized:	I. 28	early report
31	Adult acute non-lymphocytic leukemia	Chemotherapy (4 agents)	II. placebo BCG + placebo	II. 13	early report
29	Squamous cancer of head and neck	Surgery (+ irradiation in some patients)	Randomized	78	early report
32	Bladder: transitional cell carcinoma	Surgery	Historical	30	early report
30	Various, 1st remission	Different types	Randomized	26	early report
30	Various, 1st remission	Different types	Randomized, placebo-treated	29	early report
30	Various, 1st remission	Different types	Randomized, placebo-treated	119	early report

\* see reference list; p.c. = personal communication; the preceding reference indicates an earlier paper on the same study

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## DISCUSSION

### *Crohn's Disease*

*Dr. D. D. Felix-Davies (United Kingdom):* When talking about the pathogenesis of arthritis and how levamisole acts, it should be remembered that there have been cases where levamisole has precipitated arthritis, two of which were reported in the BMJ last year (*Br Med J* 2: 555, 1977).

This is a third case, of Crohn's disease observed over a number of years and after starting levamisole a bilateral symmetrical polyarthritis occurred, acute in onset with an inflammatory exudate and polymorphs in the biopsy specimen. The case improved with cessation of levamisole, although there was still some synovial hypertrophy 12 weeks later. A variety of immunological tests have been done and proved to be negative. Additionally, we measured immune complexes but again with negative results. Why levamisole should be associated with the onset

of arthritis in Crohn's disease is a moot point, but Trabert has published in *The Journal of Rheumatology* that levamisole can act, in adjuvant arthritis, to increase the severity if given at a certain time after the adjuvant. We suggest that in Crohn's this may be acting as an adjuvant occasioning precipitation of arthritis.

*Dr. J. M. A. Wilton (United Kingdom):* It is attractive to speculate on the arthritis in the patients with Crohn's when you remember Segal and Murphy's original paper about defective leucocyte migration in such patients. Levamisole has been shown both *in vivo* and *in vitro* to reverse this defect of migration and it may be that the arthritis in the Crohn's patients is polymorph mediated as many people think rheumatoid arthritis may well be. So speculation about T-lymphocyte adjuvency or immune complex deposition may not be correct.

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## **Adjuvant treatment with levamisole in cancer**

### **A review of experimental and clinical data**

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#### **Introduction**

As levamisole (Figure 1) has continued to raise much interest lately and as clinical data have started to emerge suggesting its usefulness in clinical cancer therapy, the authors felt that the time had come to prepare a survey of the available data in this field. In view of the very large amount of information available about this substance, the authors have decided to focus primarily on *in vivo* data whilst only briefly referring to *in vitro* work and findings about the intimate mechanism of action of the drug.

Since levamisole is thought to affect the cancer process through its effects on host defense mechanisms, the present introduction will primarily try to put levamisole treatment into perspective by broaching two facets, i.e. the interrelationships between the



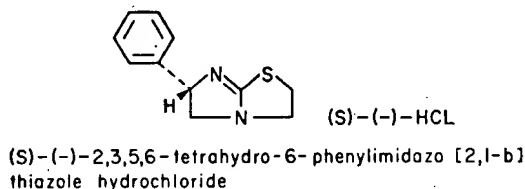


Figure 1. Chemical structure of levamisole.

tumor-bearing host and his cancer, and the basic properties of levamisole and its use outside the cancer field. Thereafter, the experimental data and, subsequently, the findings in the human will be discussed.

#### *The host defense against cancer and the rationale of tumor immunotherapy*

There is little doubt that the tumor and the host affect each other detrimentally. The original concept of "immunological surveillance" (21), which attributes a decisive role to immunity in the elimination of incipient neoplastic disease has been questioned by some authors (130), but its role, at least in the prevention of lymphoreticular malignancies, seems clear (135) and the concept, therefore, is still supported by others. Also, evidence is accumulating that the host is able to mount an immune response to the tumor-associated antigens although, in the clinical setting at least, such an immune response often fails to destroy the tumor. One of the most striking features of antitumor immunity, therefore, is its relative incapacity to eliminate the tumor when confronted with large numbers of malignant cells.

A specific antitumor defense response has been repeatedly demonstrated in experimental tumors (10, 11, 51, 72, 81, 112, 181, 192) and in man (34). Not only lymphocytes, but also, and perhaps primarily, macrophages play a decisive role in these host defense responses (52, 146, 194). Moreover, the inhibitory activity of an intact immunity on metastasis formation may be ever more important (1, 22, 52, 172).

However, it is not only because of its relative nature that the antitumor immunity fails in preventing clinical neoplasias from growing and disseminating. Recent evidence strongly suggests that the tumor is immunosuppressive in itself, affecting both the tumor-oriented immunity (1, 10, 11, 72, 81, 96, 112, 181, 192) and the more general host defense mechanisms (15, 18, 36, 37, 48, 61, 71, 74, 82, 165, 167, 191). Fortunately, the latter immunosuppression is reversible after elimination of the tumor burden (10, 11, 81, 92, 96, 112, 181, 191, 192). Apart from this systemic immunosuppression, the tumor may induce local immunosuppression in its micro-environment either non-specifically (94), through the phenomenon of "immunorepulsion" (50), or specifically (1). Perhaps, this mechanism may explain both the "sneaking through" of an incipient cancer and the development of metastatic foci.

There appears to be little doubt that a deficient immunity is a bad prognostic sign in cancer patients (33, 74, 86, 89, 92, 155). The fact that cancer-associated immunosuppression can be alleviated by eliminating the tumor burden, therefore, suggests that the prognosis of a cancer patient may be improved if his immunity is restored to normal at the critical moment, i.e. after reduction of the tumor bulk, but before the residual cancer has again grown to a volume which can no longer be controlled by immunological means. This reasoning provides the background of current immunotherapy of cancer. However, the hypothesis becomes further complicated in that classical anticancer treatments are

immunosuppressive in themselves. This is generally well-known for cytostatics—it has even been argued that these immunosuppressive drugs may actually increase the tumor growth if they fail to inhibit the cancer (159). It also occurs with irradiation [here the suppression may even be longlasting (111, 168, 176, 198)] as well as with surgery and/or anesthesia (20, 31, 32, 33, 35, 45, 65, 80, 93, 95, 103, 120, 147, 148, 156, 188, 189). The correction of therapy-induced immunosuppression, therefore, may be considered an additional aim of cancer immunotherapy.

From the above, it is clear that the strategy of immunotherapy is to back the host defense mechanisms at a time when the tumor mass is greatly reduced and the residual tumor cells ought, therefore, to be most susceptible to immunological attack. At this time, the host has already been sensitized by tumor antigens, which have been present for a considerable length of time. In view of this, non-specific cancer immunotherapy, instead of aiming at selective immunological stimulation, tries to make existing immune responses more effective (157) by interfering with the homeostatic control of the host defense mechanisms, with the ultimate goal of tipping the balance in favor of greater internal control of the cancer growth. This goal has been pursued with some success (89, 115, 116, 154, 197) by using vaccines and other bacteriological products. The advantage of levamisole resides in the fact, that it is a synthetic chemical and that it restores immunity to normal rather than stimulating it above normal, in contrast to other immunostimulants. Also, the fact that it can be given by mouth makes the drug clinically more attractive.

#### *The mechanism of action and other non-oncological properties of levamisole*

Levamisole, an imidazo-thiazole derivative, has been widely used as a dewormer for years. Although a few observations had already pointed to an improved host defense in levamisole treated subjects (85), it was not until late 1971 that the immunotropic properties of this substance were described (137). Since that time, evidence has been steadily accumulating suggesting that levamisole does not seem to affect normal immunity, but that it preferentially restores cellular host defense mechanisms when the latter are deficient, therefore, levamisole may be called an anti-nergic chemotherapeutic agent (173). It is beyond the scope of this review to cover the data relating to the intimate mechanism of action of levamisole. Nevertheless, it is perhaps important to mention here that some investigators believe that levamisole tips the cAMP/cGMP balance in favor of the latter in thymus-dependent lymphocytes and in phagocytic cells (8, 68, 133). This change is expected to further lymphocyte-mediated toxicity (170), lymphocyte motility (152), the T-lymphocyte rosetting phenomenon (67) and the motility and activity of phagocytic cells (84, 142).

As a new potential adjuvant treatment modality, it is important that the toxicity of levamisole therapy does not outweigh its benefit. In this respect, levamisole seems to have an advantage over other immunotherapeutic modalities. Indeed, the only major side-effect of levamisole is an allergic and sometimes spontaneously reversible agranulocytosis (173) which mainly occurs in patients treated for rheumatoid arthritis but which has rarely been observed in cancer patients. Other side-effects seem to be: dysosmia and metallic taste (100); nausea, emesis and anorexia (5, 100); nervousness and sleep disturbances (5); and skin rashes (100); however, the incidence of these adverse experiences may hardly differ from that observed with a placebo (5). It is also of interest that, in a surgical adjuvant setting, the incidence of non-disease mortality with levamisole was comparable to that of a placebo (5).

It is not surprising that levamisole, in view of its broad impact on host defense mechanisms, has been tested in a wide range of conditions which are associated with a deficiency of these functions. Indeed, particularly promising results have been obtained so far in recurrent and chronic infections, postviral anergy, and rheumatic diseases (173).

### Experimental oncology

#### *Method and scheme*

The purpose of this section is to review, in a first part, the available *in vivo* data from all animal experiments; a second part will deal with a few individual experiments which have studied particular aspects in this field and, finally, the conclusions will aim at summing up the data and finding a basis for extrapolation to the human cancer problem in the clinic.

The starting point for the overall analysis, which covers all *in vivo* data that came to the attention of the reviewers before June 1976, has been the idea that, if levamisole is an effective drug, the available animal data should provide us with indications of the type of efficacy and with a theoretically ideal animal model for testing this and analogous substances. However, the analysis was hampered by several factors, and particularly, by the knowledge that there are no well-established animal models for cancer immunotherapy available (23). Also, the fact that investigators often use a limited number of animals for their experiments makes a statistical approach less useful in evaluating the results. Moreover, it appeared that the reproducibility of the data leaves much to be desired as, in fact, has also been the case with other immunotherapeutic agents. It is known, however, that such a reproducibility may not only be dependent upon easily controllable factors such as sex and age of the animals (97), but also upon factors which are often not communicated in the reports. The latter factors may include the composition of the animals' food (25, 26, 88, 125) and of the atmosphere in their cages (70), stress (56, 129, 134, 158), the growth rate of the tumor (usually only the mean survival time, MST, was available as a rough estimate of this variable), the possible superinfection of the tumor inoculates by viruses, and the percentage of macrophages present in such inoculates (this percentage, which is of particular relevance to the behavior of the tumor (194), may fluctuate considerably from one tumor to another).

In view of these problems, it was decided to keep the evaluation as simple as possible and the following procedure was finally adopted:

- (1) Each experiment was considered separately and labeled "positive" or "negative" according to the presence or absence of a beneficial trend in favor of levamisole. This was one of the very few approaches which allowed us to look at the data in a standardized way.
- (2) All doses have been recalculated as related to the weight of the animals. In the few experiments, where the racemic compound tetramisole (consisting of equal amounts of levamisole and the inactive dexamisole) had been used, the dose of tetramisole was halved and thus expressed as mg of levamisole; and as repetitive dosing did not seem to affect the end-result, the doses discussed are those administered per application.

#### *An overall analysis*

Three animal species have been studied: mice, rats and hamsters. About 600 experiments have been reported in mice, but considerably less in the other two species (12, 27-29,

40-44, 53-55, 59, 60, 63, 78, 83, 87, 105, 106, 110, 117, 128, 138, 139, 149, 161, 164, 169, 174, 177-179).

The dose-effect relationship was only analyzed in mice, because the numbers were considered insufficient in the other species. Also, only in mice did it prove possible to analyze the importance of the growth rate of the tumor by means of the MST in controls. Other topics for analysis were the effect on growth of the primary tumor as compared to that on metastasis formation, and the effect of levamisole as an adjunct to classical anti-cancer treatment as compared with levamisole monotherapy. The former could not be analyzed in rats as the data were insufficient in number, whereas the latter was not analyzed in hamsters for the same reason. In mice, we have considered only those experiments in which the effects on the growth of primary and of metastatic tumors were studied synchronously in the same model, and the experiments in which levamisole as an adjuvant therapy was compared with its use in monotherapy.

### 1. Studies in mice

(a) *Dose-response relationship.* Regardless of the characteristics of the experimental tumors and of the test setting, the best results seem to be achieved with doses above 2.5 mg levamisole per kg bodyweight (Figure 2, thick line: the sharpest rise in the curve occurs when the dose exceeds 2.5 mg/kg). With the latter doses, 101 out of 364 experiments have turned out positive against only 35 out of 218 with the lower doses, and this difference is very significant ( $P < 0.01$ , Fisher exact probability test, two-tailed). Moreover, increasing the dose beyond 5 mg/kg does not seem to result in a better effect and doses above 10 mg/kg may even reduce the effect.

(b) *Slow-growing versus fast-growing tumors (as defined by their MST).* Seventy-nine out of the 407 experiments with fast-growing tumors yielded positive results, but 57 out of 175 experiments with slow-growing cancers were considered positive. This difference is highly significant ( $P < 0.001$ , Fisher test, two-tailed). Moreover, slow-growing tumors seemed

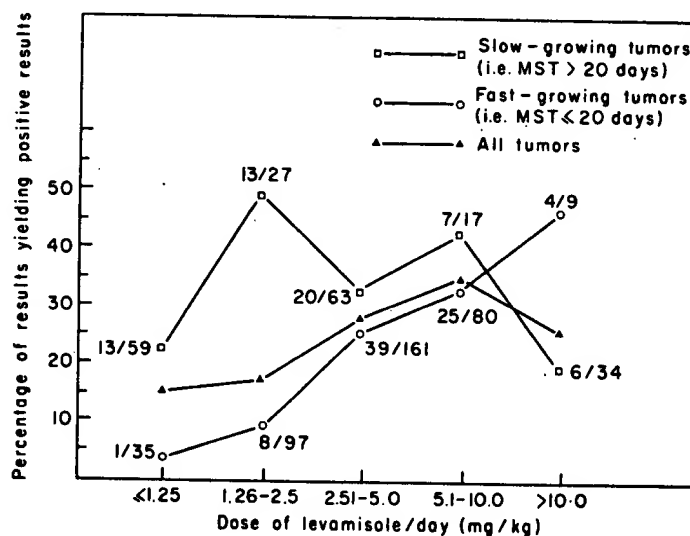


Figure 2. Effect of levamisole in mice on transplantable tumors with different growth rates.

already affected by doses 1.26–2.5 mg/kg in contrast to fast-growing ones which needed higher doses (Figure 2).

(c) *Primary versus metastatic tumors* (Figure 3). As a rule, the primary tumor was not or only marginally influenced (14 positive experiments out of a total of 78) whilst a much clearer effect was found regarding the concomitantly studied metastasis formation (30 positive experiments out of 78). This difference, again, is very significant ( $P < 0.01$ , Fisher test, two-tailed). Except for the conclusion that doses  $\leq 1.25$  mg/kg are too low, this data does not provide more information on a dose-response relationship, as altogether too few experiments have been performed with doses 1.26 to 2.5 mg/kg.

(d) *Monotherapy versus adjuvant treatment* (Figure 4). If any effect at all is to be expected from levamisole monotherapy, the dosage will probably be very critical, as only with doses 2.51–5.0 mg/kg a reasonable number of positive experiments have been found (9

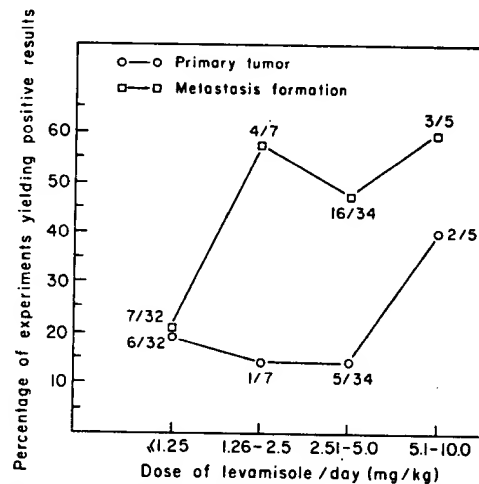


Figure 3. Effect of levamisole on metastasizing transplantable tumors in mice.

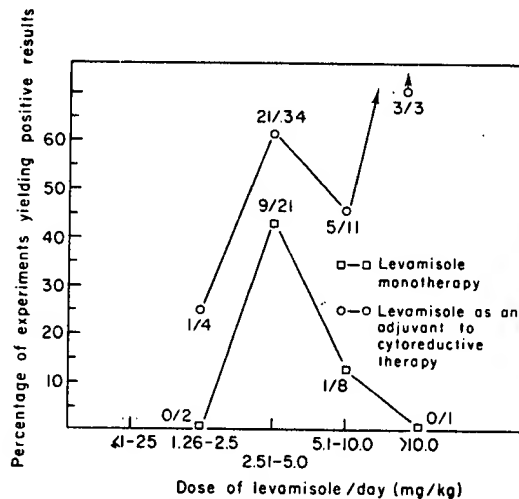


Figure 4. Effect of levamisole on transplantable tumors in mice: monotherapy as compared with adjuvant treatment.

out of 2 in contrast to only 1 out of 11 with the other doses). However, for each of the dose ranges used, the effect of adjuvant treatment (as compared to controls treated with classical anticancer therapy) with levamisole yielded much more positive results than that of levamisole monotherapy (as compared to untreated controls). Therefore, adjuvant treatment proves significantly ( $P < 0.03$ , Fisher test, two-tailed) superior to monotherapy.

## 2. Studies in rats and hamsters

(a) *Monotherapy versus adjuvant treatment in rats.* Five out of the 10 "adjuvant" experiments gave positive results as compared to only 6 out of 43 "monotherapy" studies, confirming the findings in mice that adjuvant treatment is superior.

(b) *Primary versus metastatic tumors in hamsters.* In 48 experiments metastasizing tumors were used: results with levamisole were considered positive in half of them, whilst only 6 of the 24 studies with non-metastasizing tumors were rated positive. This confirms the more pronounced effect of levamisole on metastatic tumors already found in mice.

### *Some individual topics*

The following sections are aimed at illustrating the above findings and at discussing a few aspects of them. The references do not, however, necessarily reflect the intrinsic importance of the papers mentioned.

#### 1. A representative model

In retrospect, only one model fulfilled the four criteria necessary to obtain a beneficial effect (i.e. optimal dose of levamisole, slow growing tumor, metastatic potential, and the use of levamisole as an adjunct to classical anticancer therapy). Therefore, we felt it desirable to give a more detailed description of this model (164).

The tumor used is the Lewis lung carcinoma, a slightly immunogenic tumor transplanted intramuscularly ( $2 \times 10^5$  cells) into isogenic C57B1/6 mice: the criteria for evaluation are: MST, weight of the primary tumor, and the number of (metastatic) lung nodules 23 to 25 days after transplantation. Levamisole (or tetramisole) was administered i.p. in the following doses: 3 mg (levamisole), 5 mg (10 mg tetramisole), and 10 mg (levamisole) per kg. Methyl-CCNU (16 mg/kg i.p. on day 7 after inoculation of the tumor) was used as a cytoreductive treatment. As no striking differences were found between the three doses of levamisole or between the treatment schedules (levamisole was administered on day 15, or 19, or 21, or on all three days), the results are pooled and presented in Figure 5; more details will be found in the original paper (164). It is clear that the best results are achieved with levamisole used after cytostatic treatment. Summing up, levamisole monotherapy may reduce the number of lung metastases in this model, but this is not associated with a prolonged survival as MST seems to be determined by the weight of the primary tumor, which remains almost unaffected by the treatment. On the other hand significant life-span prolongation and even cures do occur when levamisole is employed in a combined chemo-immunotherapeutic approach.

#### 2. The combination of levamisole with other immunotherapeutic approaches

Experimental data on such combinations are scarce. Also, part of them come from immunoprophylaxis studies which are of questionable relevance to the clinical situation

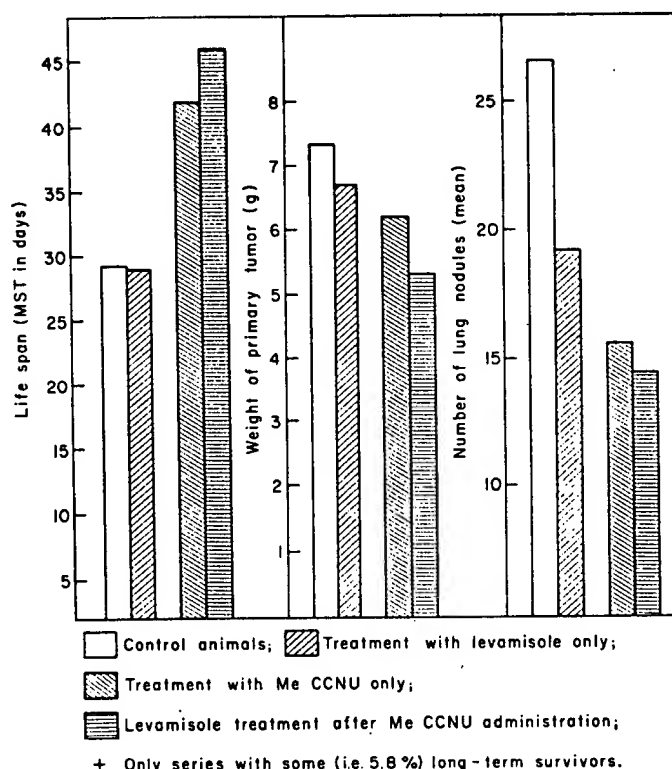


Figure 5. Effects of levamisole in the Lewis lung tumor model.

(23) and only two experiments have been reported in which treatment with levamisole combined with another immunotherapeutic modality was used concomitantly with cytoreductive therapy.

(a) *Immunoprevention studies.* Two groups of investigators (43, 164) reported that levamisole (2.5 or 3 mg per kg i.p.) used in conjunction with immunization by irradiated L 1210 leukemia cells was capable of increasing survival after inoculation with live L 1210 in CDF<sub>1</sub> mice.

(b) *Immunotherapy studies*

*Mice.* Two research groups (7, 162, 174) studied the use of levamisole combined with another immunomodulator. Beneficial effects were obtained with the combination with BCG in glioma 203G1 in C57B1 mice (174) and with *C. parvum* in the Lewis lung tumor and the P815 mastocytoma in the mouse (7). The combination of immunization with irradiated cells and levamisole treatment has also been studied in three experimental models (53, 164). No beneficial effects were obtained in the B16 melanoma of C57B1/6 mice and adenocarcinoma 15901 of A-mice (53), whilst significantly more long-term survivors were found in the L 1210 leukemia model in CDF<sub>1</sub> mice (164).

*Rats.* One group (174) reported on the combination with BCG in Sato's lung cancer transplanted to Donryu rats. The effects were not clearly superior to those of BCG or levamisole alone. Another group (178) obtained no evidence of efficacy of a triple immunotherapy modality, consisting of BCG, irradiated tumor cells, and levamisole, in the MC-1 cancer model.

(c) *Surgery plus combined immunotherapy.* Beneficial effects have been observed in the Lewis lung tumor model in C57B1/6 mice when *C. parvum* (0.7 mg/mouse i.v.) was given 1 day before surgery (day 10 after inoculation) and followed by levamisole (3 mg/kg i.p.) 9 days after the operation (7).

The combined use of irradiated cells and levamisole in a surgical model is also reported in the section on the antimetastatic potential of levamisole.

### 3. Tumor enhancement

Much concern exists amongst some clinical oncologists regarding a possible activation of tumor growth by immunotherapy. This paradoxical phenomenon has been observed in several animal systems and has been discussed at length in the literature. It should be stated, however, that the clinical relevance of animal models showing tumor enhancement with immunotherapy can often be questioned. Below, an overview is given of activated tumor growth reported with levamisole in animal models.

In individual experiments with Moloney virus-induced rhabdomyosarcomas in BALB/c mice, levamisole treatment seemed to be associated with an accelerated growth of the primary tumor, but this was not significant and even counterbalanced by a greater number of animals which showed inhibited tumor growth with levamisole. The investigators, therefore, concluded that levamisole had no effect on the primary growth of these tumors (86).

In an allogeneic model using L 1210 leukemia transplanted to C3H mice, an increased number of tumor takes was observed with levamisole treatment. This effect was associated with an elevated level of serum blocking activity (106).

Levamisole treatment starting before or on the day of tumor inoculation was also reported to stimulate primary tumor growth in an allogeneic system with an adenovirus-12 induced tumor of mice transplanted in hamsters (128).

Finally, the administration of levamisole before transplantation of adenocarcinoma 15091 to syngeneic A-mice resulted in an earlier appearance of tumors but did not affect the overall incidence or course of tumor growth, and the administration of the drug 3 to 5 days before i.v. injection of melanoma B16 into C57B1/6 mice resulted in an increased incidence of pulmonary nodules. Such nodules were significantly reduced if levamisole was given 1 day before the i.v. inoculation in the same model (53).

One more observation of facilitation of primary growth is reported in the next section (131).

### 4. The antimetastatic potential of levamisole

Although a limited antimetastatic effect may be observed with levamisole monotherapy in some models (Figure 5), its clinical use seems more promising in conjunction with cytoreductive therapy. Such anticancer treatment consists not only of cytostatics but may also include surgery, as illustrated by a recent study which only came to the knowledge of the reviewers after the completion of the overall analysis of the animal data and which, therefore, was not included in that analysis. In this study, the effect of levamisole was studied on the metastasizing behavior of B16 melanoma in syngeneic C57B1/6J mice (131). Only the data with 8 mg levamisole per kg will be discussed here as the other doses used (0.08 and 0.8 mg/kg) are considered too low as compared to the effective dose in mice found in this review. The findings may be summarized as follows. Levamisole monotherapy



given 10 or 11 days before surgery enhanced metastasis formation, but the simultaneous use of levamisole and immunization with  $10^4$  irradiated tumor cells resulted in a significant suppression of lung metastases. However, surgery combined with levamisole (on the day of operation or the following day) produced a significant reduction of the number of metastases whilst the number increased by combining the three therapies, i.e. levamisole, immunization, and irradiated cells.

Another interesting observation was reported in the allogeneic Fortner's melanotic melanoma no. 1 in Syrian golden hamsters (83). When given to surgically untreated animals, levamisole produced only a minimal prolongation of survival. However, when the drug was orally administered (three doses were studied  $\geq 2.5$  mg/kg) to hamsters showing local melanoma recurrence following surgical excision of the primary, tumor regression was observed in two-thirds of the animals and about one-half of these proved tumor-free at autopsy. These data, obtained with macroscopic tumor recurrence in an allogeneic system, may suggest that the antimetastatic effect of levamisole observed in syngeneic models is, at least in part, due to the regression of microscopic foci of the disseminated tumor.

#### 5. *The combined use of levamisole and of cytotoxic treatment*

Immunotherapists are often concerned about the concomitant use of any immunotherapy with cytotoxic agents, as they fear that the latter treatment may kill immunologically competent cells which have been activated by the immunotherapy. This fear is further substantiated by experimental data showing that the positive effects of BCG disappear if cytostatic drugs are given after the BCG administration (6, 107, 108, 121) and by similar observations with *C. parvum* (38, 121). Two studies may suggest that levamisole resembles the other types of immunotherapy in this respect. In one series of experiments, it was found that the effectiveness of levamisole is maximal at a time when BCNU-induced remission of a syngeneic murine leukemia occurs, and, coincidentally, during the period of BCNU-induced immunosuppression (29). The results seem to indicate that levamisole helps in overcoming depleted immune cell compartments in BCNU-treated animals and, therefore, that the use of levamisole before or synchronously with cytostatics is expectedly far from optimal. More direct evidence that the simultaneous use of levamisole and cytostatic drugs may be injurious comes from another study by the same group. In this study, it was found that levamisole given 5 to 8 days after BCNU treatment resulted in a significant recovery of DNA synthetic capacity of spleen cells, whereas treatment with levamisole the first day after BCNU resulted in an additional inhibition of DNA synthesis (196). Timing of levamisole treatment after cytostatic chemotherapy could, therefore, be rather critical and this seems to hold true for other types of immunotherapy as well (47, 122, 195). The studies on the effect of levamisole on spleen DNA synthesis seem all the more relevant as it has been shown that levamisole causes an earlier return of lymphoid cells in the splenic lymphoid follicles and lymphoid elements in the red pulp of BCNU-treated leukemic mice and since it has been suggested that the tumor depletes the tumor-directed lymphocytes in the spleen and that removal of the tumor bulk results in a restoration of the concentration of antitumor spleen lymphocytes (112).

#### *Comments and conclusions*

Animal models, in general, are only of limited relevance to the clinical problem and cancer models certainly are no exception to that rule. Therefore, extrapolations from these

models to the human cancer problem ought to be handled cautiously and their validity has to be proven by clinical experiments. It is within the framework of these considerations that the following tentative conclusions are put forward:

- (1) *The effect of levamisole is dose-dependent.* In mice, the efficacy seemed almost limited to those experiments in which doses in excess of 2.5 mg/kg were used. The latter dose, therefore, may be considered the threshold dose. Increasing the dose above that level did not produce superior effects, whilst with high doses (exceeding 10 mg/kg) there was some indication that the effect was again diminished. If the same holds true for the human being with his relatively smaller body surface, the threshold dose would be expected to be lower. No better results are to be expected above this threshold dose.
- (2) *Levamisole is more effective on slow-growing tumors.* This may be understood by the relative nature of the antitumor immunity as a fast-growing tumor will probably reach a critical mass more quickly beyond which immunity is no longer able to eliminate the cancer. In view of this, it will be of interest to find out whether fast-growing tumors in the clinic will be susceptible to levamisole treatment or not.
- (3) *Levamisole preferentially affects metastasis formation.* The importance of selective antimetastatic treatment for the clinic is obvious (163). However, the point is not only to prevent a tumor from disseminating (e.g. during surgery), but also, if feasible, to eliminate existing but clinically undetectable micro-metastases in combination with the eradication of the primary tumor. As already reported, an animal model using an allogeneic tumor (83) has provided data which suggest that levamisole treatment may be able to achieve this. In fact, the results from the syngeneic Lewis lung model (164) are very suggestive of such an effect as well, since micro-metastases are usually present in this model as soon as the tumor becomes clinically detectable.
- (4) *Levamisole as adjuvant treatment.* Expectations are that the future of specific systemic immunotherapy will lie in its role as part of a combined modality approach (23). The mechanisms by which immunotherapy, and classical anticancer treatment, provoke tumor cell loss appear different and are, therefore, hopefully complementary. However, the combination of levamisole with other anticancer therapies is subject to some uncertainty. This is particularly true for the combination with cytostatics, which have qualitatively and quantitatively different interactions with the host defense mechanisms. At present, it might be best to initiate levamisole treatment as soon as possible after achieving the maximum antitumor effect, but not before the immunosuppressive effects of the cytostatic therapy have started declining. A similar problem may arise with radiotherapy, though this type of treatment is possibly less deleterious to the immunity as it is a regionalized treatment modality. As surgery-associated immunosuppression is probably not due to a cytotoxic effect, there seems to be no contraindication to starting levamisole treatment before the operation.
- (5) *Tumor enhancement unlikely with levamisole.* As summarized above, the only reproducible models where enhancement has been proved were allogeneic systems. Such systems are probably not representative of what happens in syngeneic conditions and possibly provide only information about homograft reactivity instead of tumor immunology. Strangely enough, enhancement with other types of immunotherapy appears also to occur preferentially in allogeneic systems

(195). In a few syngeneic models, enhanced tumor growth has been reported as well. Even if these observations can be confirmed (to our knowledge no data on their reproducibility are available), their relevance to the clinical situation is still open to question as only enhancement of primary tumor growth has been observed in these models whilst no cases of enhancement have been reported with levamisole used in an adjuvant treatment modality.

### Clinical data

As the collection of clinical data is obviously bound to proceed much more slowly than that of experimental findings, clinical experience is more limited. This part will mainly consist, therefore, of two sections; the first one will deal with immunological effects of levamisole in cancer patients, the second one will summarize the data obtained in studies with prognostic evaluations.

#### *Effects on the immunity of cancer patients*

Several aspects of the machinery controlling the host defense mechanisms have been studied with levamisole, both *in vivo* and *in vitro*.

The first paper (180) reporting the effect of levamisole treatment in cancer subjects was concerned with delayed skin hypersensitivity to dinitrochlorobenzene (DNCB). All patients in this study had advanced solid cancers and all were tested, after having been sensitized, with four doses (25, 50, 100 and 200  $\mu\text{g}$ ) of DNCB. The effect of levamisole treatment, 150 mg daily for 3 days, was compared with a similar but untreated control group of patients. The results are summarized in Figure 6: 11 out of the 20 levamisole-

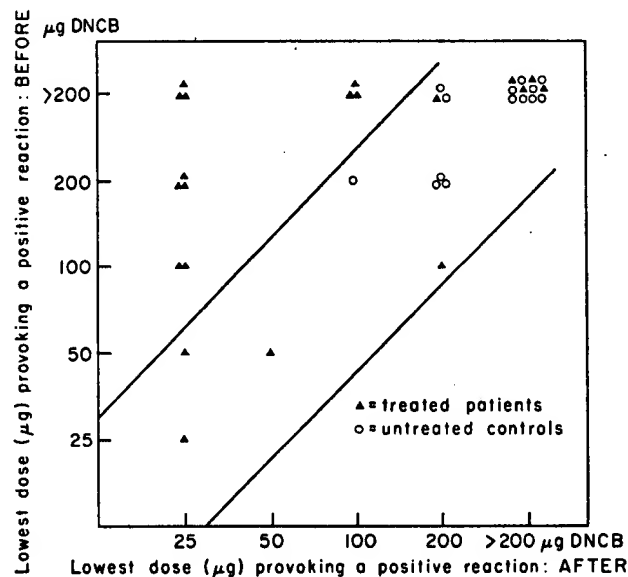


Figure 6. Effect of oral levamisole treatment on skin test reactivity to various doses of DNCB. Patients whose reactivity to DNCB did not show a clinically significant change are found in the diagonal area from lower left to upper right.

treated patients showed a clinically significant increase of reactivity whilst no such increases were observed amongst the controls. Moreover, if patients with a good initial reaction (i.e. positive with 25 or 50  $\mu$ g) are excluded, it is seen that 11 out of the 17 bad responders had increased their reactivity with levamisole treatment. In the same study, similar results were obtained by testing skin reactivity to 5 I.U. of purified protein derivative of tuberculin (PPD) used as a recall antigen. Several investigators have confirmed these findings (14, 75, 77, 98, 99, 143, 144, 182-184, 193) although not all anergic patients became skin test positive after treatment and a few investigators have reported negative findings as well (46, 76). The latter may probably find an explanation in the type or stage of the cancer studied and/or in inadequate dosing (5). In this respect, it is noteworthy that prolonged continuous treatment with levamisole was found less effective than intermittent treatment (183, 184) and that increasing the weekly dose of levamisole, but still using a fortnightly treatment schedule, resulted in increased effects (144).

The number of circulating thymus-derived lymphocytes as measured by the E-rosette formation techniques were restored both *in vivo* and *in vitro*, in patients with a reduced T-lymphocyte level (16, 98, 113, 133, 186, 187, 193); the same applies to the use of mitogen-induced lymphocyte stimulation techniques (24, 62, 64, 98, 99, 118, 140, 141). In one experiment, levamisole was first added *in vitro* and subsequently given as an oral treatment to patients with Hodgkin's disease. Most of them were clinically free of disease after appropriate treatment (133): the *in vitro* increase of E-rosette forming lymphocytes could be reproduced by the *in vivo* treatment and the effect of this treatment (150 mg for three days) persisted for at least two months in several patients although the effect had already leveled off within one month in others. In contrast, no change or even some decrease of B-lymphocyte levels was observed in the same patients.

The absolute lymphocyte count may increase with levamisole treatment, perhaps, especially in irradiated patients (98, 143, 144), though this phenomenon does not always prove reproducible (133), and after chemotherapy (175). In this context, it may also be worth mentioning that levamisole treatment has been reported to activate the bone-marrow restoration after chemotherapy (101, 102), showing some similarity with animal studies on the repopulation of the spleen after BCNU administration (124, 196).

Other interesting observations with levamisole in cancer patients are the induction of interferon (123), the increase of pre-existing humoral and cellular antitumor immunity (73, 153, 193), the restorative effect on defective monocyte chemotaxis (19, 126) and on spontaneous monocyte migration (132), and the increase in total hemolytic complement activity and in  $C_3$  levels (185).

Therefore, in conclusion, levamisole appears to beneficially affect several functions which conceivably play an important role in the defense mechanisms of the host against cancer.

#### *Coupling immunological changes with prognosis*

Only a few investigators have attempted to evaluate the prognostic implications of levamisole-induced immunological changes in cancer patients. Their experience is summarized below.

In one study (182) on patients with advanced solid cancers, it was found that the pre-levamisole skin test reactivity to DNCB did not correlate with the survival time, whilst both in placebo controls and in post-levamisole patients weak DNCB-reactivity was associated with shorter survival time and good responses with longer survival.

In another study (143, 144) it was found that an increase in reactivity to DNCB and to *C. albicans* extract was only seen in irradiated Stage III breast cancer patients who remained free of disease during follow-up examinations.

Similarly, an enhanced lymphocyte reactivity to PHA appears to be associated with prolonged survival of intractable cancer patients (140, 141).

Apart from this, two reports are available on the use of levamisole in a patient with (angio-) immunoblastic lymphadenopathy. This disease or these diseases (57, 58, 104, 109, 119) find themselves at the border of the hematological malignancies as they are often associated with mortality, though pathologists tend to classify them as non-malignant.

One patient with angio-immunoblastic lymphadenopathy had been treated with levamisole (150 mg daily 3 days per week for 6 weeks): within 4 months, his immunological parameters had normalized although he initially suffered a severe immunological unbalance (evidenced by very low T-cell activity and by high levels of B-cells); his fever disappeared, his bodyweight increased and the size of the lymph nodes decreased. The patient was still doing well one year after the treatment had started, although his prognosis had initially been considered quite bad (13).

Similar observations, i.e. clinical improvement associated with a correction of a pronounced immunological imbalance, have been made in a patient with immunoblastic lymphadenopathy (49). This patient was treated with 150 mg of levamisole daily for two consecutive days every week. Duration of follow-up in this second case was shorter, however, than in the previous one.

#### *Studies evaluating the prognosis in cancer patients treated with levamisole*

##### *1. Uncontrolled observations*

Although dispersed clinical investigators appear to have treated a limited series of patients in private pilot studies, most of this experience has remained unreported and can, therefore, not be dealt with in this review.

In a pilot study involving 23 patients with diverse stages of malignant melanoma (nine patients had already developed metastases), no arrest of tumor growth nor tumor regression could be observed when levamisole (150 mg daily for two consecutive days every week) was combined with other immunotherapeutic treatments and cytostatic drugs in disseminated cases, whilst the short duration of the follow-up precluded an evaluation of the other cases (145).

In another study (150), 57 patients with advanced solid tumors were treated with levamisole (150–450 mg daily continuously) after all seemingly appropriate classical anticancer treatment had failed. No beneficial effects were seen in the 45 patients who had been treated for less than 3 months. In 9 of the 12 patients who had been treated for a longer time, beneficial effects were reported by the investigators. These were as follows:

- (a) Arrest of tumor growth in 4 patients (3 with bronchial carcinoma and 1 with cancer of the ovary): for 3 of them, this arrest lasted for more than 10 months.
- (b) Delayed growth of lung metastases in 1 patient with malignant melanoma and an estimated prolongation of survival by 8 months.
- (c) Temporary reduction in size of metastases in 3 patients (1 with malignant melanoma and 2 with breast cancer).

- (d) Complete regression of a bronchial tumor in 1 patient after 8 months of treatment.

The investigators give no description of the diagnostic criteria used in their patients. All of these 9 patients had previously received radiotherapy and 7 of them had also received cytostatic treatment. Late effects of the latter therapies, the capricious clinical course of some of these diseases and the fact that hidden factors might have been present at the start which could explain part of the observed phenomena, were not discussed as possible explanations of the findings by the investigators.

Another pilot study of levamisole monotherapy in 21 patients (50 mg t.i.d. for 3 days every 2 weeks in most patients) yielded the following results (190):

- (a) No objective effect in 3 children with disseminated neuroblastoma.
- (b) Of 4 adults with disseminated malignant melanoma, 3 failed to respond, but in the fourth a black subcutaneous mass reduced in size from 4 to 2 cm diameter over 2 months before regrowing.
- (c) Nodules of breast carcinoma recurrent on the chest wall after surgery failed to respond in 4 out of 5 patients, but in the fifth patient, a 1 cm diameter nodule disappeared over the course of 3 months and remained impalpable for another 4 months before reappearing.
- (d) In 4 out of 9 patients palpable axillary nodes found shortly after simple mastectomy failed to respond. In 4 patients, the nodes reduced to at least half their original size for 3, 2, 2 and 1 months. In the remaining patient, nodes became completely impalpable for 9 months before reappearing.

Although a greater effort to measure the effects has been made in this study, the lack of several data in the report precludes a sensible appreciation of the results, as was the case in the previous paper.

Finally, clinical results appeared favorable in a group of 24 patients with malignant glioma or metastatic brain tumor, who received 3.5 mg levamisole per kg every day or every other day for 2 weeks. In some of these patients, levamisole was given as a monotherapy, in others it was used as an adjunct to Me-CCNU treatment 100 mg every 6 weeks (175). However, a straightforward appreciation of the finding in this case, is also hampered by the lack of indispensable data.

## 2. Controlled clinical investigations

(a) *Levamisole as an adjunct to surgery in lung cancer.* Four interim reports (3-5, 171) have already been published on the results of a continuing collaborative multicenter study in resectable lung cancer. The latest of these papers (5) reports results obtained in the first 178 of the 200 anticipated patients selected for this experiment. This is a randomized placebo-controlled double-blind study. One tablet, containing either 50 mg of levamisole or a placebo, is given three times daily on three consecutive days every fortnight for 2 years after surgery. These 3-day courses are started 3 days before surgery in an attempt to prevent operation-related immunosuppression. The end-points of the study are recurrence and carcinomatous death. So far, the most important findings from this study have been that:

- (1) The dose of levamisole is probably too low for patients whose weight is greater than 70 kg, as the beneficial effects found in the study are confined to the lower weight category of patients who had received approximately 2.5 mg/kg/day. This difference has been present throughout the four analyses made so far al-

though it had been overlooked at the time of the first interim evaluation (3, 4). In the latest analysis (5), actuarial calculation of disease-free survival in the patients who had been adequately dosed, was 91% with levamisole and 80% with placebo 6 months after the operation. These figures had dropped by 12 and 24 months to 83% and 71% respectively for levamisole and to 64% and 49% for placebo. Similarly, striking and statistically very significant differences in cancer mortality were seen between the two treatment groups.

- (2) The effect of levamisole did not appear to be clearly related to the initial skin test reactivity nor to the tumor histology, but the drug proved more effective in patients having more extended tumors at the time of surgery.
- (3) There is probably a differential effect of levamisole on the type of recurrence, as intrathoracic relapses were only marginally less with levamisole whereas the drug proved especially effective in controlling blood-borne secondaries. This is illustrated in Figure 7.

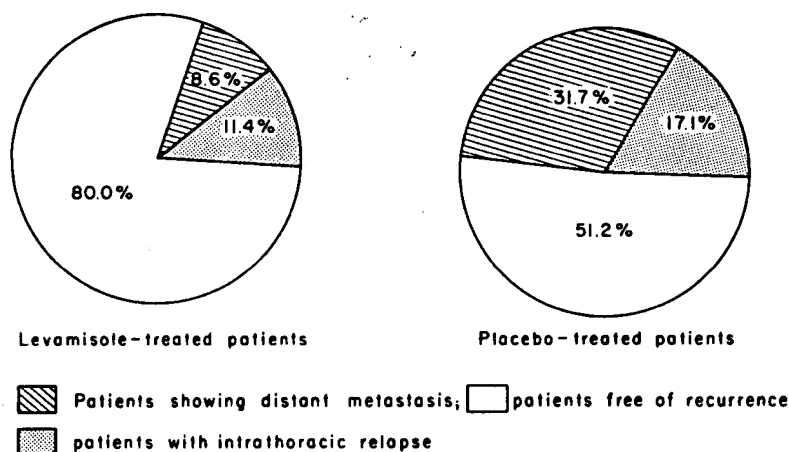


Figure 7. Site of first recurrence in adequately dosed patients. Double-blind study in resectable lung cancer.

It is proposed that the dose of levamisole be adapted to the patients' weight (estimated dose: 2.5 mg/kg daily) or to their body surface (approx. 100 mg/m<sup>2</sup>/day) in future trials and that careful attention in such trials be paid to the initial tumor load and the site of the first recurrence as this information may give clues to the mechanism of action of levamisole and to its field of clinical application.

(b) *Levamisole with surgery in malignant melanoma.* In a double-blind study (160) still in progress, 132 patients with primary or recurrent malignant melanoma are involved. The patient material breaks up into six clinical and pathologic subgroups of patients with different prognostic variables. Following their operation, the patients are randomized within each subgroup to receive levamisole or placebo. The treatment is given as a single 150 mg-dose for three consecutive days every other week for 2 years, but the delay from surgery till the initiation of double-blind treatment is not stated. When the first interim analysis was performed (160), no difference was found between the levamisole-treated patients and the controls for either disease-free survival, or duration till first visceral recurrence (however, the incidence of such metastases was unusually low, i.e. about 25% in the total population only), or crude survival. The authors insist that this is only a

preliminary report and that the number of patients followed for a prolonged time is relatively low.

(c) *Levamisole and irradiation of breast cancer.* One controlled prospective study in irradiated breast cancer patients has been reported (143, 144). Forty-eight patients, most of them menopausal, were included. Forty-three of them could be evaluated. All patients presented with Stage III breast carcinoma (UICC classification). First, these patients were treated with radiation therapy (by a cobalt source: 4000 rad to the chest wall, 4000 rad to the supraclavicular area, and 3000 rad to the posterior axillary field) over a mean period of 2 months. After completion of this treatment, the patients were alternately assigned to either the control group or the levamisole-treated group. The dose of levamisole was 150 mg orally per day during three consecutive days every fortnight until there was evidence of progressing disease.

All 43 patients (20 treated with levamisole and 23 controls) have been followed for at least 21 months after completion of the radiotherapy. The disease-free interval was significantly longer in the treated group as compared with the controls, since the median interval was nine months for the control group and 25 months for the treated patients as calculated by the actuarial method (life-table analysis). Whereas in the lung cancer study reported above, the site of the first recurrence was evaluated, in this study the sites and frequency of all early metastases (i.e. recurrences which were present when relapse was first established after the irradiation) were compared: no differences were found between the two groups except for lung metastases which proved more frequent in the levamisole-treated group.

Similarly to the disease-free interval prolongation, a significant difference in survival was found. Ninety per cent of the levamisole treated patients were still alive 30 months after irradiation treatment in contrast to only 35% of the controls (figures calculated by means of life-table analysis). The data have not been analyzed according to the weight of the patients.

Another interesting finding was that an increase of skin-test reactivity to DNCB and *C. albicans* appeared to be associated with an increased chance of disease-free survival. This is shown in Figure 8. Also, the radiotherapy-induced depression of the absolute lymphocyte-count which was present before the initiation of the levamisole treatment was completely restored by month 6 in the levamisole group whilst significant suppression was still detectable in the control group by that time.

Based upon the findings of this trial, it was suggested by the authors that patients who develop recurrent disease in less than 12 months may have had insufficient time to benefit from levamisole treatment, and that conversion of a negative DNCB skin test to a positive one is a good indicator of therapeutic efficacy. Therefore, higher doses of levamisole (300–600 mg on the treatment days) until strongly positive responses to DNCB are attained are recommended for future trials. Once conversion has been observed, the dose can be lowered for further maintenance purposes.

(d) *Levamisole and cytostatic chemotherapy in leukemia.* Information about the use of levamisole in combination with cytostatics is scarce. The reasons for this may be that most tumors are treated by surgery and/or radiation therapy when first detected and that the animal data leave us with some unresolved questions concerning the use of levamisole in clinical practice. Nevertheless, some work is in progress in this area.

The first interim results from a double-blind placebo-controlled Danish study in acute myeloid leukemia (AML) which is in progress at present have recently been reported (17).



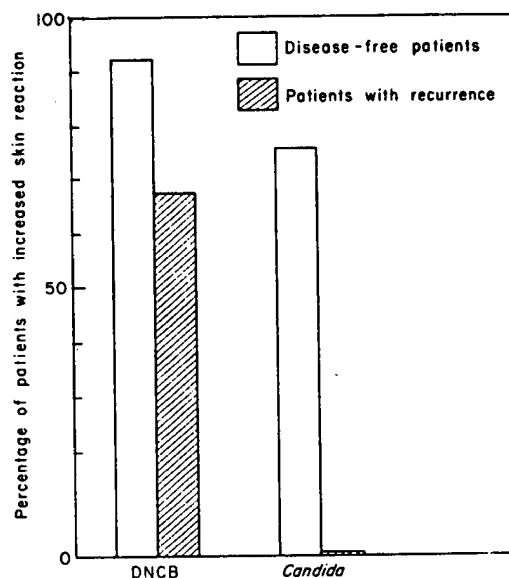


Figure 8. Controlled study in irradiated Stage III breast cancer.

This is a two-center trial in adult AML-patients presenting a first AML-episode. The two departments use the same induction treatment, i.e. 5 successive days of 24-h infusion of cytosine-arabioside ending with daunomycin on the sixth day. One of the two departments used a slightly smaller final dose of daunomycin, but supplemented the cytosine-arabioside treatment with thioguanine, given concomitantly. This treatment is repeated after 10 to 14 days until complete remission (which was eventually obtained in 50% of the patients). After this induction treatment, consolidation treatment is given and thereafter, the maintenance treatment. Details of both the consolidation and the maintenance treatment are given in Figure 9.

On top of this, patients are randomized at the end of consolidation treatment to receive either placebo or levamisole (2.5 mg/kg daily for three consecutive days every second week) on a strictly double-blind basis. This treatment is given during the week preceding and that following the "cytostatic" week (Figure 9).

In March 1976 (i.e. when the first interim analysis was performed) 24 patients had been randomized and followed for at least 6 months. Twelve patients had been treated with levamisole and 12 with placebo and the two treatment groups proved very comparable. The median duration of remission was 16 months with levamisole and only 10 months with placebo. The difference was not significant as could be expected in view of the limited number of patients. On the other hand, the graph produced by actuarial analysis of the duration of remission with placebo completely coincided with that of the 49 patients in the L-6 protocol of Clarkson (30) who used a treatment closely resembling the cytostatic regimen used in the placebo group. Therefore, in all probability, the favorable trend in this levamisole study does not seem to be caused by an unusually bad performance of the placebo patients.

(e) *Levamisole and radiotherapy in miscellaneous cancers.* A simple study was reported (39) in which consecutive cancer patients referred to a radiotherapy department were alternately allocated to levamisole therapy, or to a reference group. Levamisole treatment (150 mg

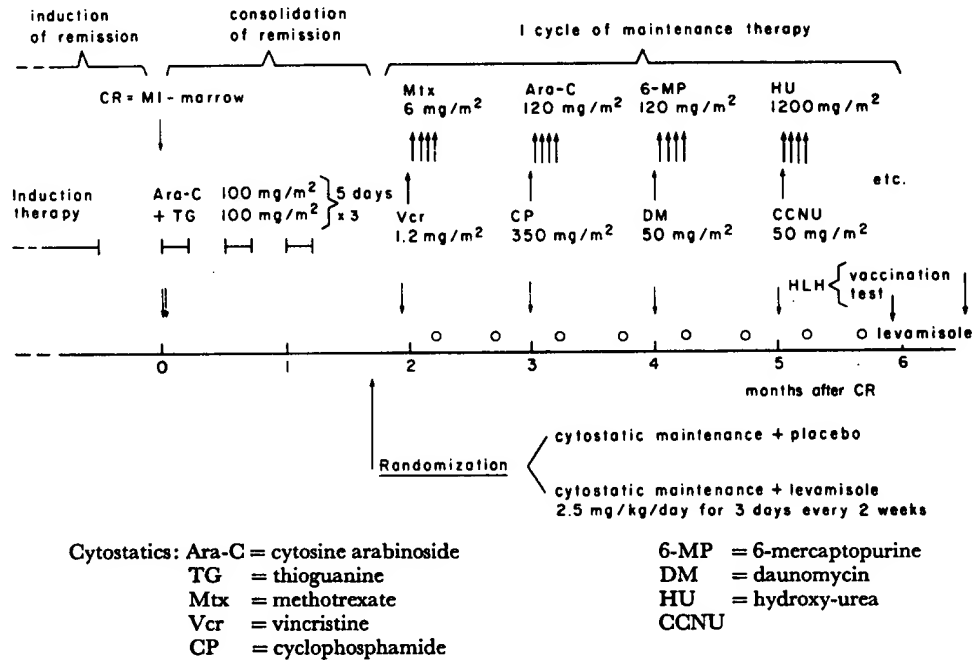


Figure 9. Treatment protocol in Danish AML study.

daily for 3 days every other week) was given in addition to appropriate radiotherapy and all patients were followed-up regardless of the effect of the irradiation treatment. In this study, 375 patients have been followed for up to 3 years (median: 2 years) from the day of admission to the trial. The data were analyzed by the actuarial method: a slight difference in favor of levamisole was found. More than 50% of the patients were still alive after 2 years: a 25% mortality was recorded after about 8 months in the reference group and about 12 months in the levamisole group; the estimated survival rate after 2 years was approximately 54% in the control patients and about 62% in the levamisole-treated subjects. Moreover, the favorable trend was consistently present in all types of cancer, except for lung carcinoma. It was most pronounced in the patients with breast cancer, who actually turned out to be the largest group. Also, the effect of levamisole in breast cancer patients appeared dependent upon the UICC-stage of the disease, as no difference was found between levamisole-treated patients and controls having Stage I or II disease, whilst the difference was most marked amongst Stage IV patients (median survival 6 months in the control group as compared with 20 months in the levamisole group).

(f) *Levamisole added to several types of cytoreductive therapy in various malignancies.* In another multicenter study which is still proceeding (66), all cancer patients who have just received remission-inducing cytoreductive therapy are included without further preselection. They are then randomized into placebo treatment or levamisole therapy (50 mg t.i.d. for two consecutive days every week) and this double-blind therapy is initiated as early as possible after the completion of the primary anticancer treatment. The patients are followed for at least 2 years or until death and carcinomatous mortality is regarded as the cardinal end-point of the study. At the time of the latest analysis (September 1976), 246 patients had been included in the study: 119 were receiving levamisole and the other 127 were on placebo treatment. Fifteen patients (i.e. 12.6%) in the levamisole group had died as

compared with 25 placebo-treated patients (19.7%). The median duration of follow-up was 5 months with both levamisole and placebo and more placebo patients had died in all of the five centers where mortality had already occurred. A breakdown of the results according to the type of tumor is not (yet) available, however.

#### *Comments and tentative conclusions*

The main purpose of this section is to compare the trends which have been found in the clinical data with those which emerged from the studies in experimental models. Moreover, an attempt is made to delineate the future position of levamisole in the anticancer treatment strategy. It is obvious that the latter part, in particular, can only be speculative.

(a) *How do the clinical data fit in with the conclusions from the animal studies?* Some clinical data (5, 143) seem to confirm the animal findings that a certain threshold dose should be exceeded in order to obtain beneficial effects. In the other studies, the importance of the dose has not been evaluated. One of the experiments (5) also suggests that the optimal dose should approximate 2.5 mg/kg bodyweight daily or about 100 mg/m<sup>2</sup> body surface. This dose, then, may be divided into two-three doses and given for three consecutive days every fortnight or for two consecutive days every week. A flexible dosage schedule, shaped to the patient's immunity, has not been worked out yet, mainly because it is not known which tests should be used for this purpose.

Thus far, the clinical data obtained from tumors with different growth rates, do not substantiate the impression from the animal studies that levamisole may be less active in fast-growing tumors, as the findings in acute myeloid leukemia (probably the fastest growing malignancy evaluated in the controlled studies) seemed promising as well (17).

The findings in lung cancer (5) appear to back the conclusion from the animal models that levamisole is more effective as an antimetastasis treatment. This feeling is not invalidated by the data of the Stage III breast cancer study (143) for two reasons. Firstly, the incidence of metastatic sites in the latter study was expressed as a percentage of the total number of patients showing recurrence and not as related to the total population treated with the drug, as was done in the lung study. Secondly, only the site of the first recurrence was evaluated in the lung study as opposed to all early sites of renewed tumor growth, regardless of the sequence of their occurrence, in the breast study. The site of the first recurrence, which occurs when the host's immune system is less suppressed by the tumor load, may be more indicative of a preferential effect on metastasis formation than the total percentage of new foci, part of which occur when the immunity is once again gravely undermined by a considerable tumor bulk. It seems, therefore, that in future studies particular attention should be paid to the location of the first relapse, though it is acknowledged that it is sometimes difficult to indicate with certainty which site was the first, as two or even more sites of regrowth may, at times, appear almost simultaneously. It may be of interest in this regard to recall the fact that other trials in lung cancer have also provided suggestive evidence that metastasis formation may be more inhibited by other types of immunotherapy than the local recurrences (127, 166).

Though controlled data with levamisole as a monotherapy are scarce, repeated contacts with several investigators who have not published their uncontrolled experience (personal communications), have clearly suggested that not much is to be expected from levamisole if used alone. On the other hand, the reported controlled data seem to indicate that levamisole is a promising agent if used as an adjunct to classical anticancer treatment, which is in keeping with the animal findings. It appears, therefore, that levamisole therapy ought to be aimed at consolidating anticancer effects obtained with other, classical

anticancer treatment. Some discussion may remain, however, concerning the modalities of these combined therapeutic approaches. As regards surgery, the available data (5), indicate that good results may be obtained if the treatment is started three days before the operation. With irradiation therapy, the problem whether the synchronous use of levamisole and irradiation might weaken the beneficial effects of the immunotherapy, remains partly unsettled as both with synchronous treatment (39) and with a sequential schedule (143) promising results have been obtained. This, however, does not exclude the possible superiority of the one modality over the other and it seems, therefore, desirable to initiate carefully designed studies comparing the two treatment approaches. In the meantime, the more cautious sequential approach might be advocated. On the other hand, much is still to be learnt concerning the combined use of levamisole and of cytostatic drugs. There are no clinical data available on the synchronous use and this modality should, perhaps, not be studied for ethical reasons, as the animal studies seem to indicate that such combinations may prove deleterious to the host. Sequential use has been studied (17) but the data, though promising, are limited. Therefore, guidelines for the use of levamisole as an adjunct to cytostatic chemotherapy are still genuinely tentative. In view of the available data, both clinical and experimental, it might be suggested that levamisole treatment be initiated as early as possible after maximal tumor reduction has been obtained, but not within the first couple of days after the last administration of the cytostatics or, if adequate immunological monitoring becomes feasible, not before the immunosuppression has started waning.

No proven cases of tumor enhancement have been reported with the clinical use of levamisole and this is in keeping with the animal data. Nevertheless, such a possible adverse effect should still be borne in mind, especially if patients with advanced disease are treated, as tumor enhancement has been reported with other types of immunotherapy in advanced cancers. However, as clinical impressions can hardly be considered conclusive in this respect there seems to be every reason to evaluate this possibility very carefully by measuring, at regular intervals, the doubling time of the tumors.

(b) *Other leads from the clinical studies.* Two controlled studies (5, 39) seem to indicate that better effects (as compared with a placebo or no treatment) are obtained with levamisole if the cancer is more advanced at the time when primary treatment is started. Also, the only negative study so far (160) was concerned with resectable melanoma, a cancer which has a small volume (as compared with other solid tumors) as long as it is still considered resectable. It may not be a coincidence that, on the one hand, levamisole is thought to correct immunosuppression rather than to stimulate the immunity (173) and, on the other hand, more advanced cancers appear to be associated with more immune depression, as can be learnt from certain animal models (181) and as has been documented with many human cancers as well. Therefore, levamisole may be particularly beneficial in more advanced cases, provided the tumor can first be clinically eradicated by classical measures, and, thus, there seems to be every reason to analyze clinical data in relation to the initial tumor load in order to evaluate whether the existing trend is correct. It does not automatically follow that levamisole is an effective treatment for the more severely immunodepressed patients with advanced cancer, as control of the tumor by the usual means seems necessary before one can expect any benefit from levamisole therapy. Therefore, and also in view of the fact that levamisole does not seem to alter the primary tumor growth in an appreciable way, the combined use of this drug with cytostatic chemotherapy in advanced cancer is not expected to produce more tumor regression than the cytostatics alone. However, as levamisole appears effective in animals in stabilizing anticancer results obtained

by conventional therapy it may seem worthwhile to evaluate whether this drug, given after tumor reduction induced by cytostatic chemotherapy, may prolong survival in patients with advanced malignancies.

The histological diagnosis, so far, has not proven to be a reliable measure to predict levamisole effectiveness. This is illustrated by the fact that trials in several malignancies have produced promising data, that the effect of the drug in resectable bronchial cancer (5) did not appear related to the histology of the tumor and that in the controlled studies where several types of malignancies have been studied concomitantly (39, 66) most of these seemed to do better with levamisole. In fact, this should not be surprising if one considers that the immunogenicity of a tumor is thought to be a characteristic of its membrane and that histological classifications make primarily use of other aspects of the cancer cell structure and of the tissue from which the cancer has originated; the possible presence of neo-antigens on the membrane of the malignant cell, on the other hand, is obviously not a criterion which is used in our current histological classifications system. Perhaps, an immunological classification of malignant diseases may prove much more useful for predicting effects of clinical immunotherapy, but this is still far from the realm of practical reality.

It is the hope of most clinical immunologists that, one day, they will have the possibility to monitor immunotherapy by measuring immunological changes. Unfortunately, the available information is too scarce to indicate the parameters which can be used for such purposes. Therefore, there is an evident need to analyze the possible relationship between clinical outcome and immunological data in prospective clinical studies with levamisole. In that regard, there is some indication that sequential skin tests (143), E-rosetting (133) and the removal of blocking substances from the lymphocyte membrane by levamisole (114) may become helpful indicators.

(c) *Other aspects.* From the theoretical point of view, the possibility exists that levamisole may exert some beneficial effects on cancer patients which are not directly connected with the outcome of the disease itself. These aspects have not been summarized in this review because they are of limited impact and, also, because there is only very little information available about them. Nevertheless, they cannot be overlooked entirely within the scope of an overview like this one.

Opportunistic infections in immunologically compromised cancer patients are apparently a badly neglected area in the clinical care of such patients, though there is ample evidence that such infections do occur in a great percentage of cancer patients, and in particular in those with hematological malignancies (2, 9, 90, 91, 136, 151, 199), and that they may be the major cause of death in 36% of such patients and a contributory factor to death in an additional 13% (2). Perhaps, some clinical data showing benefit from levamisole monotherapy in patients with advanced disease (182) should be interpreted by keeping this in mind. Direct evidence, however, that levamisole may protect such patients from infections is not yet available, and it may be worthwhile to initiate studies evaluating this aspect. In the same context, it might be useful to assess the subjective feelings and general performance of levamisole-treated patients with advanced cancer, e.g. by means of the Karnofsky scale (200).

Another topic of interest is whether levamisole enhances bone marrow reconstitution after cytotoxic treatment, as has been suggested by one group of investigators (101, 102). If true, this effect may enable a shortening of the intervals usually observed between courses of cytostatic treatment, allowing a more aggressive, and hopefully a more effective anticancer therapy.

### Summary

Animal and human studies of adjuvant treatment with levamisole in cancer are reviewed and discussed. From the animal data it is concluded that the activity of levamisole is dose-dependent, more effective on slow-growing tumors, affects metastasis formation, preferentially is best when levamisole is used as an adjuvant to the usual cytoreductive treatments and that tumor enhancement is not expected. Clinical findings are put into perspective of the animal data and the most appropriate clinical situations are indicated.

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### Addendum

When the manuscript of this review was almost finalized, two controlled studies evaluating the use of levamisole following cytostatic chemotherapy in advanced solid tumors were reported. In these studies, one in breast cancer (79) and the other in malignant melanoma (69), levamisole was found not to alter the response to cytostatics as compared with the response observed in patients receiving no immunotherapy (79) or a placebo (69). On the other hand levamisole treatment appeared to prolong tumor stabilization and tumor remission duration and to increase lifespan in patients who had experienced beneficial effects from their cytostatic therapy. Also, the effect of levamisole seemed quite comparable in the breast cancer patients with that of BCG immunotherapy.

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